**SOMFlow**

**BD FACSCanto II Standard Operating Procedure**

**QUICK GUIDE: START UP, EXPERIMENTAL SETUP, AND SHUT DOWN**

**INTRODUCTION**

This BD FACSCanto II flow cytometer SOP should help you operating and cleaning the flow cytometer to ensure its optimal working condition. Please read these instructions carefully and apply them appropriately. Maintenance, long cleaning procedures, and calibration of the flow cytometer will be done by Dr Siying Ye. If you have any questions regarding the flow cytometer or your experimental setup or when the flow cytometer is not working as it should, please contact [Dr Siying Ye](mailto:siying.ye@deakin.edu.au).

**PROCEDURE**

1. **START-UP - The first user** **of the day.**
   1. Check that the MilliQ water, FACSClean and Sheath fluid tanks are full
   2. Check the waste tank is empty. Add 250 ml of bleach to the empty waste tank.
   3. Check that the FACS Flow Cart is on.
   4. Turn on the computer. Log in to Windows as the Operator. The password is “BDIS”.
   5. Double click the FACSDiva icon to open the instrument acquisition software. Log in to the software.
   6. Turn on the instrument system power by pressing the green button on the left side of the instrument.
   7. On the main tool bar select **Cytometer > Fluidics Startup** and follow the prompts from the software.
   8. On the bottom of the Cytometer window the time is indicated for remaining warm-up time for the laser, and there are four indicators. Three green indicators for FACSFlow, FACSClean, and Shut Down Solution (full = green, red = level too low, change fluids) and one black indicator for Waste (empty = black, red = full, empty Waste fluid tank).
   9. The flow cytometer is now ready to run samples, start cleaning procedures, and for calibration.
2. **CLEANING AFTER EACH RUN – ALL USERS** 
   1. In DIVA, add 3 new tubes and name “BLEACH”, “RINSE”, and “WATER” for recording cleaning.
   2. Place a tube of 5 mL of 50% bleach (WHITE KING) on the SIT (tube holder). RECORD for 5 minutes on **HIGH**.
   3. Place a tube of 5 mL of PBS on the SIT. RECORD for 5 minutes on **HIGH**.
   4. Place a tube of 5 mL of MilliQ H2O on the SIT. RECORD for 5 minutes on **HIGH**.
   5. Click on the Remove Tube button on the Acquisition Dashboard and remove your tube while holding the SIT all the way to the left. Let the SIT go back to the middle position, and the needle is cleaned automatically. Export your data to your group folder.
   6. Export your data to your group folder and transfer to your own hard drive ASAP.

\*\*DATA THAT IS OLDER THAN 6 MONTH WILL BE DELETED.

* 1. Log out or close software.
  2. Empty Waste Tank down the sink with running water.
  3. Clean up any spills, throw away any garbage and take your belongings with you.
  4. **Check the instrument schedule to determine if you are the last user of the day. If you are the last user of the day, continue with fluidics shutdown procedure.**

\*\*Deviations from SOP (INCLUDING FAILURE TO RECORD CLEANING) that result in instrument downtime or inhibit the next user from typical use will incur extra charges.

1. **SHUT DOWN – The last user of the day**
   1. Click on “**Instrument>Fluidics Shutdown**” this will open a small text box where you should confirm the Fluidics Shutdown by clicking OK.
   2. When fluidics shutdown is complete, shut down the FACSDiva software, shut down the computer.
   3. Shut down the flow cytometer by pushing the green button on the left side of the flow cytometer. When the flow cytometer shuts down it will make some noise (depressurizing the system), this is normal.